Microtubule dynamics: **The view from the tip**

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Recent studies have suggested that proteins found at the tips of microtubules in vertebrate cells may play an important role in intracellular membrane transport processes. Evidence from fission yeast indicates that such proteins can also regulate microtubule dynamics.

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In cell biology, the location of a protein can be an important indicator of potential function, but directly demonstrating a specific hypothesized function is often difficult to achieve. In the last year, papers from several groups [1–5] have reported the identification of a number of proteins which specifically concentrate in transient clusters at the tips of microtubules in vertebrate cells. Given the functions that have already been described for many of these proteins, such clusters could serve a number of purposes, including promoting interactions with the plasma membrane, preparing local sites on microtubules for subsequent organelle binding, or regulating microtubule dynamics itself. Alternatively, they could simply represent the endpoint of plus-end-directed microtubule-based transport. Genetic evidence from fission yeast [6] now suggests that Tip1p, a likely ortholog of one of these proteins — the mammalian protein CLIP-170 — regulates microtubule dynamics in a very specific manner, which ultimately may allow microtubules to find target sites for polarized growth at the ends of the cylindrical fission yeast cell.

Over ten years ago, Rickard and Kreis [7] identified CLIP-170 (see Figure 1) as a microtubule-binding protein in HeLa cells, and showed it to be localized inside cells to small patches correlating with the position of microtubule ends. Rickard and Kreis [7] suggested that CLIP-170 might be involved in regulating microtubule dynamics. Subsequent experiments demonstrated that CLIP-170 is required for the binding of endocytic carrier vesicles to microtubules *in vitro* [8] . A role in intracellular membrane transport would be consistent with the intracellular localization of CLIP-170 (see below), but its *in vivo* physiological function has remained uncertain.

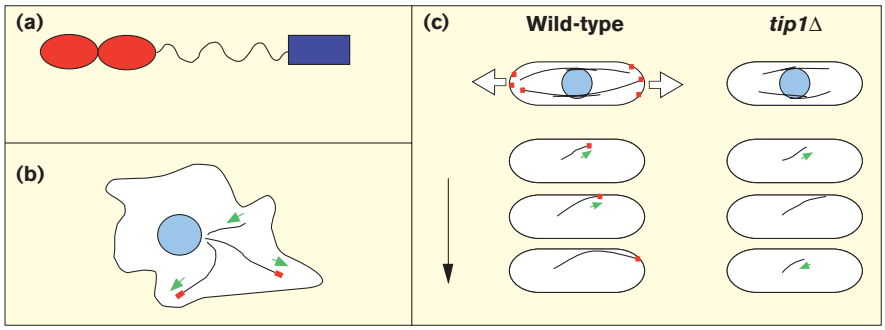
More recently, CLIP-170 was produced in tissue culture cells as a fusion protein with the green fluorescent protein (GFP), and found to exhibit several remarkable dynamic behaviors in association with microtubules [1]. Fluorescence videomicroscopy of live cells showed that GFP–CLIP170 is localized specifically to growing microtubules (see Figure 1): a 1–3 μm 'stretch' of GFP–CLIP170 fluorescence follows the extreme tips of growing microtubules, and when microtubules shrink or microtubule dynamics is paused by drug treatment, GFP–CLIP170 rapidly dissociates from the microtubule end. In addition, fluorescent speckle analysis [9] has suggested that, rather than actively translocating along the elongating tubulin polymer via a motor protein, GFP–CLIP170 may maintain its localization at the microtubule tip through a kind of 'treadmilling' behavior, exchanging with a cytoplasmic pool.

The best explanations that have been proposed thus far for this behavior are that either GFP–CLIP170, or a short-lived modified form of it, can recognize a specific transient conformation of tubulin polymer found only at the growing end [10], and/or CLIP-170 actually copolymerizes with tubulin [11]. Supporting this view is the observation that the amino-terminal microtubule-binding domain of CLIP-170 (see Figure 1), which shows the same dynamic behaviors *in vivo* as the full-length protein, is highly similar to sequences within cofactors B and E, proteins known to be involved in tubulin folding [1,11].

Similar dynamic behavior in living cells has now also been seen for other proteins previously described to be localized near the tips of microtubules by immunofluorescence (see [12]). When fused with GFP, the adenomatous polyposis coli (APC) protein, product of a tumor suppressor gene mutated in many colorectal cancers, moves along microtubules as granular aggregates [3]. When microtubules start shrinking, the aggregates detach and slowly move inward, remaining as aggregates, in a movement that is likely linked to centripetal transport of the plasma membrane cytoskeleton; such detached granules can also rebind to growing microtubules that pass by. This behavior is slightly different from that seen with GFP–CLIP-170, whereas EB1, a protein which can bind to APC and also independently associates with microtubules, exhibits a behavior nearly identical to that of CLIP-170 [2].

Emerging from these experiments is thus the notion that a microtubule tip cluster, consisting of several different proteins, may be assembled at growing microtubule ends, and this view has been strengthened by the observation that EB1 interacts with both cytoplasmic dynein intermediate chain and dynactin [13]. Cytoplasmic dynein acts as a microtubule-based motor for the directed movement of many membranous and non-membranous cargoes within

Figure 1



(a) Schematic diagram of CLIP-170 modular organization. CLIP-170 has two amino-terminal microtubule-binding domains (MTB; red), a coiled-coil central region that allows dimer formation, and a carboxy-terminal region involved in cargo binding (blue) [27] . Homologs such as Tip1p and Bik1p (see text) have the same organization, but have only one copy of the microtubule-binding domain. **(b)** Association of CLIP-170 with growing microtubules in a tissue culture cell. The nucleus is shown in blue. Growth and/or shrinkage of dynamic microtubules (black lines) is indicated by green arrows. CLIP-170 (orange marks) is found at the tips of growing microtubules only. **(c)** Microtubule steady-state organization and dynamics in wild-type fission yeast and the *tip1Δ* mutant. The nucleus is shown in blue, and Tip1p in orange. Microtubules (black lines) tend to reach the cell ends in wild-type cells but are shorter in the *tip1Δ* strain. Large arrows indicate polarized growth, at cell ends; this can be aberrant in the *tip1Δ* mutant. A model for the time-dependent behavior of an individual representative microtubules is shown below. Tip1p localized to growing microtubule tips prevents microtubule catastrophe after contact with the cell cortex, allowing microtubules to grow all the way to the cell end. In the *tip1Δ* mutant, cortical contacts in the cell middle lead to catastrophe.

cells, and this is facilitated by dynactin. In accordance with their functions, both dynein and dynactin have a widespread intracellular distribution, but careful observations have shown dynactin, and perhaps dynein as well, to colocalize with CLIP-170 at microtubule tips [4,5]. Moreover, dynactin colocalization with CLIP-170 depends on a carboxy-terminal domain of CLIP-170 that is thought to be directly involved in binding cargo [5,14] (see Figure 1).

The sum total of evidence, including functional links to dynein and dynactin, suggests that microtubule tip clusters do play a role in membrane transport [4,5], but the role of specific proteins in this process is still not entirely clear. Moreover, it remains an open question whether there may be other properties associated with microtubule tip clusters, such as the original hypothesized regulation of microtubule dynamics. Here fission yeast steps in, with new evidence from Brunner and Nurse [6] that a homolog of CLIP-170, Tip1p, contributes to the regulation of microtubule dynamic instability, by preventing premature microtubule catastrophe until microtubules reach the end of the cell.

Tip1p, which shares several conserved motifs as well as overall structural organization with CLIP-170 (Figure 1), was identified in a screen for fission yeast cDNAs the overexpression of which leads to deviations in polarized cell growth. Deletion of *tip1+* is not lethal, but *tip1Δ* mutants, like Tip1p-overexpressing cells, can display aberrant cell shapes. Previous work has shown that microtubules are important in maintaining cell shape in fission yeast [15], and one of their roles is to target an end-marker protein, Tea1p, to the ends of the cell [16]. Interestingly, cytological analysis has revealed that *tip1Δ* mutants have abnormally short microtubules, and thus fail properly to target Tea1p to cell ends, which helps to explain the morphological phenotype of *tip1Δ* mutants. Short microtubules have also been observed after deletion of a homologous gene, *BIK1*, in budding yeast [17] .

What is the reason for short microtubules in the *tip1Δ* mutant? Several groups have used GFP-tagged tubulins to follow different aspects of microtubule dynamics *in vivo* in fission yeast ([6,18–22] and my unpublished data). It is now clear that interphase microtubules, which are nucleated near the surface of a centrally positioned nucleus and span the length of the cell (Figure 1), are very dynamic structures, with half-lives of a few minutes. Interconverting between growing, shrinking and pausing phases, they show essentially all of the aspects of dynamic instability seen in higher eukaryotes. One striking feature of fission yeast microtubules is that they tend to pause as they contact cell ends before converting to a shrinking phase (known as 'microtubule catastrophe'). It is hypothesized that this paused state may be involved in the targetted delivery of components to the cell tip. Thus, for efficient delivery it would be important to have a mechanism by which microtubules carrying growth polarity-regulating components reach the end of the cell prior to initiating catastrophe.

This is precisely what Tip1p appears to do. In wild-type cells, when growing GFP-tagged microtubules contact the cell cortex, they usually continue growth towards the cell end before undergoing catastrophe (Figure 1). By contrast, in *tip1Δ* mutants, contacts with the cell cortex in the middle of the cell lead to microtubule catastrophe; catastrophe that does not involve cortical contact does not appear to be affected in the *tip1Δ* mutant. Immunofluorescence experiments show that, like CLIP-170, Tip1p is localized to microtubule tips, and probably specifically to growing microtubule tips. Thus, one can imagine a mechanism by

which microtubule tip-localized Tip1p prevents catastrophe of a growing microtubule upon contact with the cell cortex, until microtubules reach the end of the cell (Figure 1). There, some unique end-identifying properties of the cortex might promote the dissociation of Tip1p, and thus subsequent microtubule catastrophe.

This work clearly indicates a role for CLIP-170 proteins in regulating microtubule dynamics *in vivo*, and homologs in other systems will need to be investigated carefully with respect to this function. Several new questions also arise, however, to be addressed both in this and in other systems. Given the important role of microtubule tip cluster proteins in membrane transport, might there be a specific contribution of membranes or membrane-bound organelles to the regulation of microtubule dynamics? A carboxy-terminally truncated form of Tip1p, which lacks the putative cargo-binding domain (Figure 1), behaves like full-length Tip1p with respect to microtubule dynamics, even though Tea1p is no longer properly targetted to cell ends [6]. Thus, it may be the case that the functions related to membrane transport and microtubule dynamics-related functions are separable in CLIP-170 proteins. Furthermore, there may be multiple mechanisms by which microtubule tip cluster proteins regulate microtubule dynamics, as mutation of a fission yeast gene encoding an EB1 homolog, *mal3*+, leads to microtubule defects [23], and in budding yeast the EB1 homolog Bim1p/Yeb1p is also involved in regulating microtubule dynamics (see [12]).

A final question concerns the dynamic association of microtubule tip cluster/CLIP-170 proteins with microtubule tips. What regulates their departure? Early work on CLIP-170 showed that its binding to microtubules may be controlled through phosphorylation, via an as yet unknown microtubule-associated kinase [24]. Identification of components involved in this aspect of regulation will be of clear interest in all systems; intriguingly, in budding yeast one possible candidate has just emerged — the 'target of rapamycin' (TOR) kinase which is involved in several pathways [25] . Inhibition of TOR by rapamycin seems to affect microtubules in a manner similar to that seen in mutants of *BIK1*, which encodes the budding yeast CLIP-170/Tip1p homolog [26]. These effects are not exacerbated in a *bik1Δ* mutant, however, suggesting that the effects of TOR inhibition may be mediated through Bik1p.

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